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Understanding the role of the perivascular space in cerebral small vessel disease

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Abstract

Small vessel diseases are a group of disorders that result from pathological alteration of the small blood vessels in the brain, including the small arteries, capillaries and veins. Of the 35-36 million people that are estimated to suffer from dementia worldwide, up to 65% have an SVD component. Furthermore, SVD causes 20-25% of strokes, worsens outcome after stroke and is a leading cause of disability, cognitive impairment and poor mobility. Yet the underlying cause(s) of SVD are not fully understood.

Magnetic resonance imaging (MRI) has confirmed enlarged perivascular spaces (PVS) as a hallmark feature of SVD. In healthy tissue, these spaces are proposed to form part of a complex brain fluid drainage system which supports interstitial fluid exchange and may also facilitate clearance of waste products from the brain. The pathophysiological signature of PVS, and what this infers about their function and interaction with cerebral microcirculation, plus subsequent downstream effects on lesion development in the brain has not been established. Here we discuss the potential of enlarged PVS to be a unique biomarker for SVD and related brain disorders with a vascular component. We propose that widening of PVS suggests presence of peri-vascular cell debris and other waste products that forms part of a vicious cycle involving impaired cerebrovascular reactivity (CVR), blood-brain barrier (BBB) dysfunction, perivascular inflammation and ultimately impaired clearance of waste proteins from the interstitial fluid (ISF) space, leading to accumulation of toxins, hypoxia and tissue damage. Here, we outline current knowledge, questions and hypotheses regarding understanding the brain fluid dynamics underpinning dementia and stroke through the common denominator of SVD.

Introduction

The umbrella term 'small vessel disease' (SVD) refers to a heterogeneous group of vascular disorders resulting from the pathological impairment of the small vessels of the brain. It is responsible for a large proportion of the cases of stroke and dementia worldwide ¹. SVD manifests in several different ways, showing various pathological, neuroimaging and clinical presentations such as stroke, cognitive impairment, dementia, physical disability and depression ²⁻⁷, may predispose to delirium, and worsen outcome after stroke ⁸. This multiplicity of clinical expressions has contributed to delays in recognising the similarities between such patients and that small vessel damage is a common underlying pathophysiology. Importantly, the prevalence of SVD is increasing and effective disease-modifying interventions, including pharmacological treatments, are yet to be found. This presents a huge social and economic burden that needs to be urgently addressed ⁹.

Pathological evidence of SVD has been recognised since the 1800s. Since then, further post-mortem studies and advanced imaging technologies have allowed the hallmarks of SVD to be studied in greater detail ^{10,11}. MRI images from patients with SVD show characteristic abnormalities, such as white matter hyperintensities (WMH), cerebral microbleeds, lacunes and enlarged PVS ^{2,12,13}. These individual imaging features of SVD are inter-related, contribute to a 'total SVD burden', and both the individual features and the total SVD burden are associated with increased exposure to vascular risk factors in adulthood (particularly hypertension and smoking), stroke risk, concurrent cognitive dysfunction plus early life factors such as lower educational attainment, lower socioeconomic status and low childhood IQ ^{13-18, 19,20}.

Clinically 'silent' neuroimaging signs of SVD can appear during ageing, and markers of cerebrovascular disease are fairly common incidental findings on MRI performed for other reasons ²¹⁻²⁴. Most cases occur sporadically, although a small proportion are caused by genetic mutations. These latter include cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), the most common monogenic SVD, and others such as cerebral autosomal recessive

arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL; ²⁵⁻³¹), with more monogenic variants being identified all the time. ³²

Although these genetic variants show phenotypic similarities to sporadic cases, the underlying cause of most sporadic SVD remains unknown. WMH are highly heritable ³³⁻³⁶, making the limited genetic associations identified so far with sporadic SVD somewhat surprising. However, recent genome-wide association studies ^{28,33,37-39}, and recent targeted studies, have identified some genetic variants associated with sporadic SVD ^{40,41}, suggesting that genetic common variants with small effect sizes but with lifelong action could increase vulnerability to various exposures in later life, leading to accumulating small vessel damage and dysfunction ^{28,41}. Since intelligence in part reflects white matter integrity, which in turn is partially determined genetically, this could partly explain the association between childhood IQ and the SVD burden seen in later life in any one individual, and the heritability of WMH.

Amongst several potential molecular mechanisms that could link genetic traits to pathogenesis, are advanced glycation endproducts (AGEs) and activation of the receptor for AGE (RAGE). AGEs accumulate during hypertension and ageing, leading to vascular stiffening and inflammation, both of which we discuss later as important known mechanisms in SVD. AGE accumulation is increased when inflammation is present, and in the presence of oxidative stress and diabetes ⁴² indicating one of several ways that adverse effects of combinations of risk factors may be much worse than might be expected from adding together the effects of individual risk factors alone. RAGE activation leads to production of reactive oxygen species (ROS) and altered gene expression ^{42, 43}. Furthermore RAGE is activated in animal models of hypertension, and inhibition of RAGE activation prevents amyloid deposition ⁴⁴. RAGE activation is believed to be implicated in vascular diseases and neurodegenerative conditions such as Alzheimer's Disease (AD; ⁴⁵) – for example RAGE expression is increased in cerebral blood vessels of animal AD models and transports amyloid β across the BBB ^{46, 47} - but there is not yet specific evidence relating to SVD or PVS dysfunction, particularly not yet in humans.

Environmental influences, such as education and socioeconomic status, appear to modify the risk of both developing imaging features of SVD and of having a stroke, while factors such as cognitive reserve may protect cognitive function against a developing burden of SVD brain damage and contribute to variability in disease expression between patients ^{14,20,48,49}. Further evidence for a complex ‘nature-nurture’ balance underlying SVD is that although genetic and vascular risk factors, particularly hypertension, diabetes and smoking, increase the risk of developing SVD, these multiple common vascular risk factors *combined* explain only a small proportion of the variance in SVD imaging features; thus they may exacerbate a predisposition, rather than being the sole cause of SVD ^{2,50}. This interpretation is further borne out by the disappointing results to date of clinical trials of vascular risk factor reduction therapies: these have not prevented recurrent lacunar stroke, cognitive decline, or made much impression on reducing SVD lesion progression (for example, the SPS3 trial ⁵¹), further suggesting that SVD-specific treatments will require other approaches ^{52,53}.

Research into the causes and pathophysiological mechanisms of SVD has been hampered by the difficulty in visualising small vessels in the human brain during life and the fact that pathology at death is often not reflective of the early disease stages ². While specific lesions such as WMH or lacunes have received much research attention, features such as the perivascular space and its relevance to brain fluid balance have only been recognised more recently. Furthermore, while much clinical research and practice has focused separately on ‘stroke’ and ‘dementia’, and thus overlooked until recently the common underlying importance of microvessels and their dysfunction, similarly, much laboratory research has focused on either the blood vessels or the neurons/glia and thus overlooked the integration between microvessels and brain tissue and the importance of the perivascular space.

To this end, a Fondation Leducq Transatlantic Network of Excellence (TNE) is now focused on understanding the role of perivascular spaces in SVD (Figure 1). Knowledge about PVS and brain fluid and waste drainage systems in health and disease is growing. How PVS become enlarged, at what stage in the progression of SVD this occurs, and what the downstream consequences are, remain

unanswered questions. Below, we discuss the role of PVS in the healthy brain, the association between disease and enlarged PVS, and propose hypotheses for the potential involvement of these enlarged spaces in the pathogenesis of SVD which are being addressed as part of the Fondation Leducq TNE programme.

Perivascular Spaces

PVS, also known as Virchow-Robin spaces, are fluid filled compartments surrounding the small blood vessels in the brain. PVS were originally named after Rudolf Virchow and Charles Philippe Robin, who individually provided detailed descriptions in the 1800s⁵⁴. While historical descriptions of PVS and their function have been controversial^{55,56}, recent advances now recognise potentially important features such as architectural differences between PVS in different brain regions in humans⁵⁷.

The current literature consensus is that PVS form a network of spaces around cerebral microvessels that acts as a conduit for fluid transport, exchange between cerebrospinal fluid (CSF) and interstitial fluid (ISF) and clearance of waste products from the brain. Indeed, a central brain lymphatic-like system has been proposed since the 1700s⁵⁸. This clearance system has been identified in both animal models and humans and is varyingly referred to as 'para-arteriolar', 'para-venular', 'paravascular' or 'glymphatic' - a term derived from the observed dependence on functional glial cells and its similarities to the lymphatic system elsewhere in the body^{59,60}.

This proposed clearance pathway has most recently been explored in experiments involving the study of movement of fluorescent tracers in rodents^{56,61-63}. However, the system of fluid drainage is not completely understood. Efflux of ISF via PVS is proposed to facilitate waste clearance from the brain, while influx of CSF from the basal cisterns or superficial subarachnoid spaces into the periarteriolar spaces is thought to not only help flush out waste but also to deliver various signalling molecules and metabolic factors required for brain function^{64,65}. However, the precise routes of fluid clearance and whether these occur passively by diffusion or undergo periods of more active exchange by convection

as a consequence of vascular pulsation, or both, are controversial⁵⁷. Fluid transport along this pathway is thought to be driven by cerebrovascular pulsatility^{60,64} (although there is conflicting evidence for this⁶⁶ and others argue in favour of diffusion). The rate and direction of fluid movement is also controversial^{56,57,67-69}. Many of these apparent differences in space function and fluid fluxes may reflect the effects of different experimental designs, closed versus open craniotomy, temperature control, and anaesthetic agents to name but a few, on the delicate intracranial haemodynamics and perivascular space systems. In addition, the ability to capture beat-to-beat variations by real time imaging in the context of differentiating between convective versus diffusion mass transport of CSF and solutes is currently limited.

The concept of this complex brain fluid and waste clearance system is controversial, as recently reviewed elsewhere⁷⁰⁻⁷². Nevertheless, this pathway appears to be important for the clearance of interstitial solutes from the brain, and is most likely vital for maintaining brain homeostasis. This view is supported by a reasonable body of human data demonstrating widening, and increasing visibility, of PVS in various presentations of SVD, stroke, dementia⁷³, systemic inflammation⁷⁴, and associations of PVS themselves with impaired cognition and poor blood pressure or glucose control^{17,75,76}. A recent small study in patients being investigated for hydrocephalus, who had gadolinium contrast injected into the CSF followed by serial brain MRI, also suggested that fluid uptake into PVS is more active in humans during the night⁷⁷. Compromised function (potentially indicated by widening of PVS, discussed below) may therefore have a negative impact on brain health⁷⁸ and be involved in conditions such as AD, diabetes, increased risk of stroke and brain injury^{79,80}. If PVS can be measured accurately and dynamically, they present a potential biomarker and novel therapeutic target⁸¹.

Enlarged PVS in disease

Possibly the earliest description of dilated PVS pathologically was in the 1800s by Durant-Fardel^{11,54}. PVS become visible on MRI when enlarged, and though they may be detected on MRI in healthy individuals, widened PVS become more frequent during ageing and when associated with pathological

alterations to the brain tissue such as with increasing burden of SVD lesions ^{73,82-86}. Interestingly, as well as WMH being highly heritable, these enlarged PVS themselves are also highly heritable ⁸⁷. Depending on the scan orientation these enlarged PVS will appear punctate or linear. PVS were defined in the STRIVE guidelines to aid the description of SVD pathophysiological features as having a diameter 'smaller than 3mm when imaged perpendicular to the course of the vessel' ⁸⁸. Most are much smaller than 3mm in diameter and there is acknowledged to be overlap between larger PVS and small lacunes about which more research is needed.

It may seem odd that enlargement of PVS, rather than shrinkage, should be abnormal, but as we discuss later, it appears likely that widening of PVS indicates obstruction by protein and cell debris and thus stagnation of fluid drainage. There is substantial evidence that enlarged PVS are abnormal. For example, they indicate increased stroke risk ⁸⁹ particularly with lacunar rather than large vessel stroke, and other SVD features particularly WMH ^{73,90}. Their presence also correlates with vascular dementia, decreased performance in measures of cognitive function in healthy older men ^{17,91}, hypertension ⁹²⁻⁹⁴, WMH ^{73,74,76} and reduced von Willebrand factor suggesting reduced vessel elasticity ⁸⁶ in SVD. PVS enlargement can also be seen in cerebral amyloid angiopathy (CAA; ^{95,96}), CADASIL ^{97,98}, is a marker of SVD ^{73,90,93,99,100} and is possibly associated with brain atrophy. Furthermore, enlarged PVS are associated with systemic inflammation ⁷⁴, blood-brain barrier (BBB) dysfunction in SVD ¹⁰¹ and with inflammatory exacerbations in multiple sclerosis (MS; ^{102,103}).

Role of enlarged PVS in SVD

PVS are considered to play a role in normal brain homeostasis, while enlarged PVS are a feature of several diseases, and are associated with SVD. How PVS become enlarged in SVD and what the downstream effects of this are remains unclear. There are numerous potential ways in which PVS are likely to be involved in disease progression, and untangling the causes and consequences from the range of evidence in the literature is a challenge to be addressed. To investigate the factors that

contribute to the enlargement of PVS, the consequences of these enlarged spaces, and the effect on clearance of waste products from the brain via the brain drainage system, four main areas of study can be identified and are discussed below and highlighted in Figure 1.

1. What are the pathophysiological causes and consequences of the expanded PVS in SVD?

We propose that expansion of PVS is likely to involve inflammation and that this in turn will result in increased oxygen consumption. Inflammatory markers are elevated in a range of vascular disorders, in ageing mice and in elderly people with cognitive decline ¹⁰⁴⁻¹⁰⁶. There is evidence for systemic inflammatory processes occurring in SVD ^{74,107,108}. Interestingly, SVD burden is increased in lupus, an inflammatory disorder associated with increased stroke risk ¹⁰⁹. Inflammatory markers are also associated with WMH, a hallmark feature of SVD ¹¹⁰. However, the role of inflammation in SVD is still to be fully elucidated ¹¹¹⁻¹¹³ and may lead to WMH development via triggering dysfunction of PVS ⁷⁴. The triggers of inflammation are unknown, but potential factors could include salt intake ¹¹⁴ and systemic inflammatory disorders such as rheumatoid arthritis ^{50,108,109,115}.

Inflammatory markers accumulate around cerebral blood vessels as shown pathologically in traumatic brain injury, intracerebral haemorrhage (ICH; ¹¹⁶) and MS ¹⁰³. Pro-inflammatory markers are associated with enlarged PVS and inflammatory cells are known to accumulate in the perivascular space ¹¹⁷⁻¹²¹. Release of inflammatory cells can cause breakdown of the extracellular matrix and affect the integrity of the BBB, along with triggering demyelination ¹²². In the stroke-prone spontaneously hypertensive rat (SHRSP), a model of SVD, inflammation is associated with impaired myelin integrity and BBB dysfunction ^{123,124}. Furthermore, perivascular macrophages are thought to be involved in AD, and may contribute to the neurovascular dysfunction seen in this disease ^{116,125}.

The precise interaction between inflammation, enlarged PVS and brain fluid dynamics is still to be determined. It is possible that aggregation of inflammatory cells in the PVS leads to remodelling and alterations in fluid clearance. Targeting inflammation may therefore present a therapeutic avenue for

SVD. In fact, reducing perivascular macrophages in the SHRSP model improved endothelial function and remodelling of the middle cerebral artery¹²⁶. Depletion of perivascular macrophages also reduces oxidative stress and endothelial function and cognitive dysfunction¹²⁷. Further studies of inflammation in rodent models of SVD, with relation to the time course of vascular alterations and fluid movement, will help elucidate the role of inflammation in SVD.

Inflammation in SVD may be linked to reduced blood flow, hypoperfusion and hypoxia¹²⁸. It is traditionally thought that structural alterations in blood vessels and reduced blood flow are central mechanisms in SVD – in fact, hypoperfusion is used to model aspects of SVD in rodents^{129,130}. In Fisher's seminal studies he described 'segmental arteriolar disorganisation' associated with lacunes, showing enlargement of the lumen and abnormalities in arterial architecture¹³¹. It has since been proposed that dysfunction of the vessel endothelial cells leads to alterations to blood vessel architecture. These changes could lead to both enlargement and narrowing of the vessel lumen, along with vessel stiffening². Vascular smooth muscle cells are also involved in blood vessel remodelling¹³², for example a narrowed lumen has also been associated with an increase in vascular smooth muscle cells in the SHR model¹³³. Further work is needed to confirm the cause and time course of vessel alterations, but multiple studies in both patients and animal models show that overall CBF is reduced, potentially as a result of these alterations. However, the exact role of reduced CBF in SVD pathogenesis is contentious due to the lack of longitudinal studies designed to illuminate causation.

Attenuated cerebrovascular reactivity (CVR) and CBF in CADASIL mouse models have been noted prior to other alterations in brain pathology, such as lacunes^{134,135}. Regions of normal appearing WM in patients with WMH can show reduced CVR, suggesting that vascular alterations may precede WMH development^{136,137}, however direct evidence for this is surprisingly scarce. WM has fewer capillaries than the cortex¹³⁸, which along with slower blood flow and presence of a watershed area may contribute to greater susceptibility of WM to hypoperfusion than grey matter^{139, 140}. However, while increased WMH are consistently associated with decreased CBF cross-sectionally, evidence to support

the assumption that decreased CBF leads to white matter damage is somewhat lacking, as highlighted in a recent systematic review ¹⁴¹. This meta-analysis draws attention to the lack of convincing evidence that reduced CBF predates WMH in humans, the data suggesting that increased WMH precede decreased CBF rather than the opposite. Interestingly, recent studies in the CADASIL model suggest that reduced CBF and WML occur independently ¹⁴². Therefore, the temporal relationship between a reduction in blood flow and progressive damage to the brain, such as WMH, needs to be clarified ¹⁴³ as a priority.

Whether reduced CBF is cause or effect, there is strong evidence that it is a factor in SVD ¹⁴¹, and as such we can hypothesise that reduced blood flow could trigger hypoxia, neuronal death and other neuropathological alterations adding to worsening of the cerebral environment ^{122,128,144}. These changes could occur alongside inflammation and demyelination because the resulting reduction in oxygen delivery could lead to activation of microglia and macrophages, triggering demyelination ¹⁴⁵ exacerbate dysfunction of the BBB ¹²² and in turn encourage enlargement of PVS. We could further hypothesise that if fluid flow along the brain drainage pathway is driven by cerebral arterial pulsatility ^{146,147}, then increased stiffness and loss of pulsatility could lead to reduced waste clearance.

While it is likely that reduced CBF and vascular dysfunction are key events in SVD pathogenesis, the temporal association between reduced CBF and SVD pathogenesis, and its relationship with enlarged PVS remains unclear. This association can be examined using a range of techniques, including two-photon phosphorescence lifetime (2PLM) measurements of oxygen delivery ¹⁴⁸, and histological measures of hypoxia ¹⁴⁹, in rodent models of SVD.

2. The role of pericytes and BBB disruption in SVD

Endothelial dysfunction is a recognised contributor to SVD ^{2,150,151}. BBB dysfunction increases with ageing, in vascular dementia, AD and with increased WM lesion load ¹⁵²⁻¹⁵⁷ and has been found to precede the development of dementia ¹⁵⁴. Furthermore, breakdown of the BBB is found in patients

with lacunar stroke ^{101,158-160}, in WMH on MRI ^{137,160-163}, in vascular cognitive impairment and dementia (VCID; ^{152,153,164,165}) and is associated with poor functional outcome after minor cortical or lacunar stroke ¹⁶⁶.

In patients with SVD, BBB leakage is also apparent in normal-appearing WM, increases together with increasing SVD-lesion burden, and appears to predict cognitive dysfunction ¹⁵⁰, indicating an important role for BBB dysfunction in the pathogenesis and clinical expression of SVD. Patients with SVD have also been found to have elevated circulating levels of markers of endothelial activation and damage ¹⁶⁷, elevated serum levels of homocysteine, an endothelial toxin and presumed risk factor for SVD ^{168,169}, and there is evidence that the association between homocysteine levels and SVD risk is mediated by endothelial dysfunction ¹⁶⁹.

Interestingly, recent genome-wide association studies identified the *Foxf2* gene region as a major risk locus for small vessel stroke ³⁹. *Foxf2* is expressed in brain vascular endothelial cells and pericytes ^{170,171}, and mice deficient for *Foxf2* develop prominent structural and functional abnormalities of endothelial cells along with a disruption of the BBB ¹⁷¹.

The cause of the BBB dysfunction is unclear, but inflammation has been indicated as a causative factor that can trigger endothelial dysfunction (³², see above). Another potential culprit is pericyte dysfunction. Pericytes are proposed to play a variety of roles within the neurovascular unit ¹⁷² including control of the dilation of capillaries ^{173,174}. Some controversy has arisen over the role of pericytes in capillary dilation ¹⁷⁵, however this could potentially be explained by the presence of subclasses of pericytes and the correct labelling of pericytes vs smooth muscle cells ¹⁷⁶. Pericytes are also involved in the ischaemic response, contracting and causing capillary constriction, thereby presenting a potential therapeutic target ^{174,176}. Animal models developed to have reduced numbers of pericytes show reduced CBF and CVR, BBB breakdown and neurodegeneration ^{155,177,178}. More recently, pericyte loss has been demonstrated to underlie white matter damage, which is associated

with both SVD and dementia ¹⁷⁹. Animals with white matter damage resulting from pericyte loss were shown to develop axonal degeneration, enlarged PVS, and functional deficits in behavioural tests.

Pericyte dysfunction has been linked to several disorders, including AD and diabetic retinopathy ¹⁷⁹⁻¹⁸¹ and they are among a number of cells of the neurovascular unit that are affected in SVD ¹⁸². Pericyte cells have been proposed to be lost in rodent models of SVD such as the CADASIL mouse model ^{183,184}, in models of cerebral hypoperfusion ¹⁸⁵ as well as in human post-mortem tissue ¹⁸⁶, although an up-regulation of pericytes has been noted in some CADASIL patients ¹⁸⁷. When pericytes are lost in SVD, nearby endothelial cells also show signs of dysfunction, and these changes together may contribute to alterations in CBF and BBB function ¹⁸⁶.

Markers of endothelial dysfunction correlate with enlarged PVS in SVD ^{86,101} and in MS ^{102,103}, indicating a relationship between enlarged PVS and BBB breakdown. We propose that in SVD, pericyte degeneration results in opening of the blood brain barrier and aggravates inflammation in PVS. These events may further compromise pericyte and PVS function triggering a vicious cycle of events.

Of course, it is not only pericytes that are affected in SVD. Oligodendrocytes ¹⁸⁸, the basement membrane ¹⁸⁹ and the extracellular matrix ^{122,190} have all been proposed to play a role. The mechanisms underlying BBB dysfunction, and underlying the association between abnormal PVS and endothelial dysfunction are yet to be determined, and could be studied by cross-comparison between specific models of pericyte loss and models of other putative SVD mechanisms such as SHRSP. The therapeutic value of BBB preservation and how this would affect the appearance of enlarged PVS and the progression of SVD is also to be tested.

3. How are PVS and brain fluid clearance affected in rodent SVD models?

Enlarged PVS are evidently an imaging biomarker for cerebrovascular disease ^{73,76,89} and could indicate dysfunction of fluid clearance due to excess accumulation (e.g. from BBB failure) or failure to drain

the usual amount of interstitial fluid (e.g. from obstructed PVS), which may further result in impaired drainage of toxins and build-up of harmful waste products ^{191,192}. Perivascular drainage is impaired in aged wildtype mice and in models of AD ¹⁹³⁻¹⁹⁵. PVS contribute to amyloid- β clearance from the brain. Normally, transvascular clearance across the BBB is thought to clear most amyloid- β from the brain (~60-85%), whereas ISF flow across PVS removes the remaining smaller fraction (~15-40%) of amyloid- β depending on whether the animal is awake ^{196,197} or asleep ¹⁹² and better amyloid- β clearance is also associated with physical activity ¹⁹⁸⁻²⁰¹. Faulty transvascular clearance of brain amyloid- β across the BBB is likely to play an important role in amyloid- β accumulation in the brain, both in human AD and animal models ^{59,202} and might explain associations between reduced physical activity ²⁰³, poor sleep patterns and increased risk of cognitive decline and dementia ^{204,205}. In CAA, AD and other disorders, dysfunction of clearance via ISF flow additionally contributes to amyloid- β accumulation. Amyloid- β deposits are therefore suggestive of dysfunction of fluid clearance ^{193-195,206-210}. In fact, amyloid- β clearance is reduced in aged mice, alongside reduced fluid transport ¹⁴⁷, indicating a potentially key role for this process in the pathogenesis of AD and CAA ²¹¹.

Fluid clearance is significantly impaired in models of stroke, multiple infarcts, diabetes and traumatic brain injury ^{79,212-215}. Closure of PVS and impaired fluid transport has been shown in a model of migraine – commonly experienced in CADASIL ²¹⁶. Ageing and brain injury may therefore impair function of the brain clearance pathway. Dysfunction of this system could contribute to interstitial oedema, accumulation of waste toxins, and trigger pathological events that could have a devastating impact on brain health. Enlarged PVS may therefore provide not only a biomarker for SVD but also indicate impaired fluid transport and waste clearance. However, it is not currently clear whether enlarged PVS result from impaired clearance of fluid, or conversely that impaired clearance occurs as a result of enlarged PVS. It is also possible to speculate that enlarged PVS may be a compensatory response, designed to improve fluid flow. While there is evidence for reduced fluid movement in models of AD, stroke and other disorders ^{79,195,212-215}, interestingly, a recent report is suggestive of

increased ISF flow in the hippocampus of the SHR model ²¹⁷ – more long-term studies are needed since increased flow may predate reduced flow and failure of fluid clearance or vice versa.

Further studies of fluid dynamics in SVD and the role of enlarged PVS in this process are therefore warranted, and clarifying potential changes to flow in SVD presents an interesting avenue of research. It can be hypothesised that fluid transport is impaired in SVD, and in vivo MRI imaging and modelling in rodent models of SVD could be used to determine its role in disease pathophysiology ²¹⁸⁻²²⁰. The timecourse of these alterations could be studied alongside investigations of the role of inflammation, hypoxia and vessel alterations to build a more complete picture of the pathophysiology of SVD, which the field is currently lacking.

4. Understanding PVS morphology and cerebrovascular function in patients with SVD

Currently, there is no gold-standard animal model for SVD. However, animal models do provide opportunities to examine certain aspects of SVD pathophysiology ²²¹. Translational research is hugely important, and combining our knowledge of SVD at the cellular and network level in animal models with advanced imaging studies in both animals and humans will help us to develop a more complete picture of the pathophysiology of SVD (Figure 2).

As discussed above, MRI abnormalities are recognised hallmarks of SVD ⁸⁸. In addition to structural changes, such as WMH, functional abnormalities indicating impaired cerebrovascular function can also be detected. CVR and cardiac pulse transmission are both altered in SVD ^{222,223}. PVS quantification in humans has been based on visual scoring to date, which has limitations. We have developed a computational method of analysing PVS structure ^{224,225}, which can calculate centrum semiovale PVS volume and numbers meaning that we can now determine whether PVS morphology correlates with makers of cerebrovascular dysfunction, such as CVR, BBB dysfunction or serum markers in SVD. As clearance via PVS has been shown to occur diurnally, with enhanced clearance during sleep via an enlargement of the ISF space ²¹⁹, it is also likely that sleep dysfunction will affect brain homeostasis via this pathway. A small convenience study in patients undergoing investigation of hydrocephalus

provided some evidence that clearance increases during sleep in humans ⁷⁷. Abnormal PVS have been observed in patients with sleep apnoea ²²⁶ and sleep disruption occurs commonly in patients with AD and other neurodegenerative conditions ²²⁷. Sleep disruption also negatively impacts amyloid deposition in mice ²²⁸. Clearance during sleep may therefore provide a novel therapeutic avenue ⁷⁸. We aim to address this by investigating the presence of SVD-related brain changes in patients with severe sleep apnoea, and determining whether treatment with continuous positive airways pressure therapy will affect the appearance of MRI and serum markers of cerebrovascular dysfunction.

As reviewed by ²²⁹, advanced imaging techniques have been essential in developing our understanding of SVD. Rating scales and improved analysis methods will aid assessment of PVS ^{73,86,224,225,230,231}, while advances in imaging technology, such as 7T MRI, will aid the study of the role of PVS in SVD and other disorders ^{85,232}. While current clinical evidence for dysfunction of the brain clearance pathway in disease is limited, newly developed techniques are being used to image fluid flow in PVS in patients ^{233,234}. However, we need to reduce variability and cement reproducibility in MRI markers by harmonising protocols and quantification methods ¹². Validation of advanced imaging techniques for use in multicentre studies provides promise for using new methods in clinical trials ²³⁵. Longitudinal clinical studies are needed to help us to fully understand disease progression ^{236,237}.

Conclusions

SVD has a complex pathophysiology with many contributing factors. A combination of vascular dysfunction, inflammation and BBB dysfunction are likely to underlie this disease and have devastating effects on brain health. However, the timing and contribution of these events to SVD pathophysiology is yet to be confirmed. MRI has provided imaging biomarkers for SVD ⁸⁸, including enlarged PVS, which correlate with SVD burden. There is recent, and increasing, evidence for the association between abnormal PVS and SVD. However, how PVS come to be enlarged in SVD and their role in the pathogenesis of the disease, is yet to be determined. Here we discussed potential pathways that could lead to enlarged PVS and the effects this may have on brain health, and present the work that is being

addressed in the Fondation Leducq funded TNE. This network aims to tackle the problem of SVD using a cross-disciplinary approach, linking findings from animal studies (both in vivo and in vitro) to studies in patients with sporadic SVD. Furthermore, we aim to provide novel insight into related pathological mechanisms by studying SVD-related brain changes in sleep apnoea patients.

SVD is a pressing issue, with a global health impact. Enlarged PVS are just one factor in this complex disorder and there are many contributing factors that are not discussed here. Furthermore, there are many suppositions in the literature that remain to be proven. By looking at each of the processes that are known to occur in SVD in detail, and providing proof for some of the assumed knowledge we may further our understanding and uncover novel therapeutic avenues for SVD.

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Figure 1: A. Enlarged perivascular spaces are key pathological features of SVD as shown on MRI from patients with sporadic SVD (Top panel, insert: PVS detected on T2 MRI), and associate with WMH (bottom panel). B. The cycle of events we believe are involved in SVD pathogenesis and PVS dysfunction, including altered blood flow, BBB dysfunction and disrupted brain fluid flow. C. Some of the key outstanding questions in SVD research, which also outline the scientific goals of the Fondation Leducq TNE ‘Understanding the role of the perivascular space in cerebral small vessel disease’.

Figure 2: Translation between preclinical and clinical findings will be facilitated by comparing and harmonising rodent and human imaging techniques, allowing the relationship between enlarged PVS on MRI (A,B) to fluid flow (C,D) to be determined. A. Clinical imaging. Top: FLAIR (L) and T2 (R) MRI shows WMH (L) form along the PVS (R, arrow). Below: T2 shows a PVS running inwards from cortex (arrow); T2* shows white matter venule (arrow) closely related to the PVS. B. T2*-weighted MRI of a normotensive mouse (top panel) and hypertensive mouse showing cortical vessels associated with susceptibility contrast probably due to thickening of the vessel wall and/or altered perivascular spaces (lower panel). C. ‘Glymphatic’ transport pathways detected by T1 MRI and Optimal Mass Tomography analysis in a rodent brain following tracer infusion into the CSF. The timecourse of fluid flow from the cisterna magna throughout the brain can be revealed using this technique D. Fluid transport in the perivascular space detected by macroscopic fluorescent optical imaging in the cortex following injection of fluorescent markers into the CSF and vasculature.

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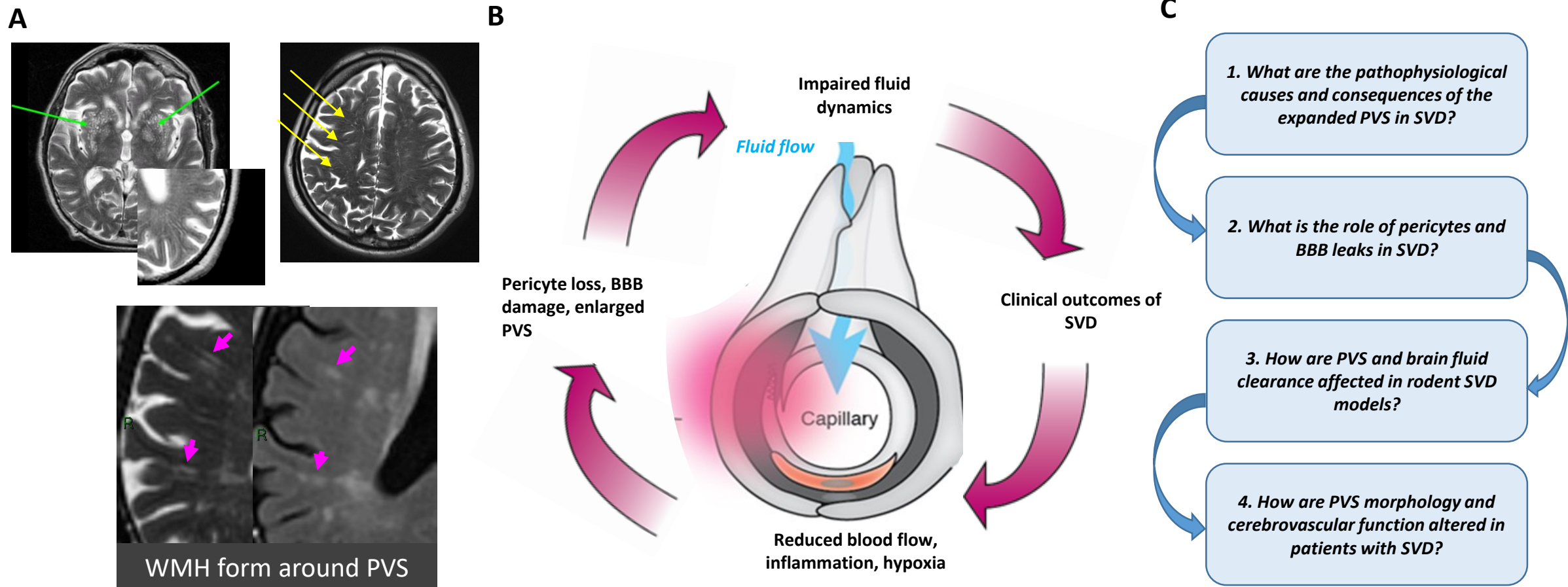


Figure 2

